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A Study to Determine the Critical Characteristics for Mice in a Newly Designed Whole Body Toxic Gas Exposure Module

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December 1993

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This project was undertaken to answer questions involved in optimizing the use of a newly designed whole body exposure chamber. To accomplish these objectives, individual groups of mice were given 10-, 20-, and 30-minute whole body exposures to moderate concentrations (near LCT 50 concentrations) of phosgene. Survival rates were recorded at 24 hours. Pertinent conclusions were based on the survival rates and were as follows. 1) The LC50 for 20-minute exposures was found to be in the range of 21 to 27 mg/m^3. 2) Spatial homogeneity of the chamber gas concentrations was satisfactory. 3) Ten-minute exposures at higher concentrations of phosgene resulted in survival rates which were consistently reproducible as thirty minute exposures at lower concentrations.

A study of the effect of animal huddling in the exposure chamber on inhalation toxicity was inconclusive, and a different plan of study for this problem is proposed

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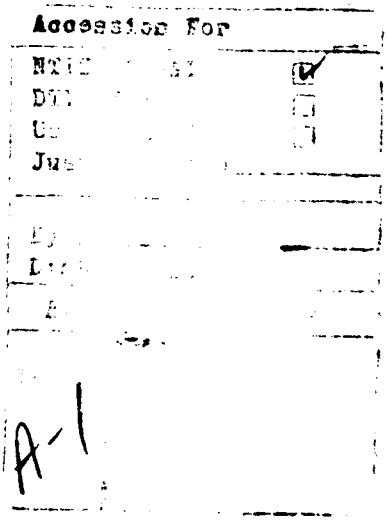

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INTRODUCTION

This project was undertaken to facilitate the use of a newly designed whole body inhalation exposure chamber. The following aspects of the chamber were studied: 1) The chamber concentration of phosgene required to result in 50 % mortality (LC50) for 20 minute exposures of mice using a 24 hour post exposure observation period. 2) The spatial homogeneity of the phosgene exposures produced by the chamber as reflected by survival rates of animals simultaneously exposed in different quadrants of the chamber. 3) The possible need for separation of animals in the chamber to prevent unequal exposures due to huddling during the exposures. 4) The question of whether or not a relatively long (30 minute) exposure to a lower concentration or a relatively short (10 minute) exposure to a higher concentration would result in more consistent survival ratios for repeated studies.

MATERIALS AND METHODS

Inhalation exposure apparatus

Inhalation exposures to toxic gas were performed as follows (see diagram, appendix). The animals were placed in a cylindrical clear acrylic chamber measuring 28 cm diameter x 25 cm height. Air or phosgene mixed with air was then supplied to the chamber. Compressed air was directed through a mass flow controller delivering 20 L/min to the exposure circuit. This airflow was used as the carrier gas for phosgene which was added at a flow rate regulated to secure the desired final concentration of phosgene. Phosgene of 99% minimum purity was obtained from Matheson Gas Products, Baltimore, as a liquified compressed gas. The rate of phosgene administration into the carrier gas was controlled manually by a needle valve to maintain the desired concentration in the chamber inlet flow, as monitored continually by an infrared spectrophotometer (inlet MIRAN) (Miran 1A; Foxboro Co., Sharon, MA). Calibration of the spectrometer was accomplished according to the manufacturer's instruction manual (Instructions for Foxboro MIRAN 1A, Foxboro Corporation, East Bridgewater, MA). The spectrometer had an analogue output, which was continuously recorded on a strip chart recorder. Volume increments of phosgene were injected into a circuit of known volume passing through the MIRAN. The concentration of the step increases in phosgene thus produced and shown on the recorder output were calculated from the amount injected into circuit and used to annotate the record. Having thus calibrated the MIRAN, the circuit was reconfigured to allow the outflow of the MIRAN to provide the inflow to the exposure chamber. The calibration record was used to determine the phosgene concentration in the carrier gas during the exposure which followed. The phosgene concentration of the effluent from the exposure chamber was also monitored by a second infrared spectrometer (outlet MIRAN) calibrated in a similar fashion. On completion of the exposure, toxic gas in the circuit was flushed by compressed air, using a flow rate similar to that of the gas mixture used for exposure. Time of exposure was recorded as time of initiating the flow of the phosgene-air mixture to the chamber subtracted from the time of initiating the compressed air flush.

Test Animals

All experiments were done using male mice, CRL:CD-1© (ICR) BR. The mice were quarantined for 5 days before use in the protocol, and maintained in plastic cages with ground corncob bedding. They were provided commercial rodent ration and water ad libitum. Initial weights were 18 to 22 gms. The average weight of the mice at time of exposure was 29.6 gms.

Procedures

To accomplish the four objectives of the study, the experimental procedures were carried out in four stages, as follows:

1) Stage I. Determination of the LC50 of phosgene for 20-minute exposures with 24-hour post exposure observation.

We studied the relation of phosgene exposure concentration to survival rate of exposed mice to obtain an LC50 concentration for this chamber for 20- minute exposures with 24-hour observation periods. The up-down method of Dixon (ref 1) was used. A series of phosgene exposures of animals was performed in which the phosgene concentration utilized for each exposure was chosen on the basis of the results of the preceding exposure. Fifteen exposures were made, using one mouse for each, with the inlet concentration of phosgene recorded for each. We subsequently found that the MIRAN monitoring the chamber inlet concentration showed an incorrect, but consistent, concentration value due to pressure buildup in the MIRAN. This pressure elevation was due to the use of high flow rates (20 - 30 liters per minute) with 1/4 inch I.D. outlet circuit tubing and connections. Because these LC50 determinations for 20-minute exposures were accomplished using the inlet MIRAN readings for phosgene exposure concentrations, we continued to use these inlet readings for the experiments of Stages II and III. We reasoned that these Stage II and III experiments depended on consistent, reproducible phosgene concentrations rather than the absolute values of the concentrations. As will be discussed, this decision to continue to use inlet concentration figures for Stage II and Stage III experiments later contributed to difficulty in interpretation of the results of the Stage II experiments.

In the subsequent determinations of 10- and 30-minute LC50 concentrations performed in Stage IV, the more accurate outlet concentrations determined by the outlet MIRAN were used. A series of experiments was performed to evaluate the relationship of the indicated (but erroneous) inlet concentration to the more accurate outlet MIRAN reading. An approximate empirical correction was thus obtained to adjust the LC50 for the 20-minute exposures of this stage of the protocol. It was found that several factors, in addition to pressure effects on the first (inlet) MIRAN, influence the difference between the recorded inlet and outlet phosgene concentrations. These will be discussed under **Results** and **Discussion**. At the completion of the study, four remaining mice were used in a brief up-down check on the calculated 20 minute LC50, using outlet MIRAN phosgene determinations. (see below)

2) Stage II. Examining the need for preventing huddling during exposures.

The probability that huddling of mice during an exposure might result in a reduction in the amount of gas inhaled when some of the animals were covered by, or compressed, by the weight of other mice was considered. To study this possibility, we compared survival rate of two groups of 24 mice exposed *en masse* in the chamber with the survival rate of two groups of 24 mice which had been separated into four smaller groups of 6 mice each by perforated quadrant dividers placed in the chamber. A significant difference between the average compartmented and un-compartmented survival rates, with survival greater in the un-compartmented studies, would suggest that huddling reduces the exposure of at least some of the animals to the gas.

3) Stage III. Testing for lack of spatial uniformity of exposures in the chamber.

We used the information obtained in 2) above to study the effect, on survival rates, of the horizontal location of the animals with respect to the floor of the chamber. The data provided a survival ratio for each of four quadrant positions, on two successive experiments. These survival ratios were examined to detect any tendency for location in a particular quadrant to associate with enhanced or decreased survival.

4) Stage IV. Determining the best exposure time.

To study the optimum exposure time for future studies, we conducted two series of experiments using, respectively, 10-minute exposures and 30 minute exposures. The object was to determine whether a longer exposure time was associated with more consistent results, as should be reflected by a reduction in variances of survival rates in repeated exposures to the same concentration of phosgene.

To determine the proper LC50 concentrations needed for these 10 and 30 minute exposures, a series of up-down experiments (ref 1) was performed as in Stage I. Phosgene concentrations were determined as <u>outlet</u> MIRAN concentrations. We used the (estimated outlet MIRAN) 20-minute LC50 found in Stage I together with Haber's rule (i.e., lethal concentration is inversely proportional to exposure time) to select starting point phosgene concentrations for these studies. Nine exposures at each of the two durations were made using one mouse for each exposure and 24-hour post-exposure observations.

Following these 10- and 30-minute LC50 determinations, eight exposures were performed for each of the two duration - concentration combinations. Each exposure utilized 12 mice. Ten-minute and 30-minute exposures were alternated. Survival results at 24 hours were determined for each exposure and a comparison made between the variance of the number of mice surviving in the 10-minute exposure groups as compared to the variance of the number surviving in the 30 minute exposure groups.

RESULTS (by stage)

1) Stage I. Determination of the LC50 for 20-minute exposures using a 24 hour post exposure observation period. (Note - phosgene concentrations for stage I are reported as <u>indicated</u>, uncorrected, inlet MIRAN concentrations)

Mouse #	CG concentration mg/m³	Result
1	75	Died
2	58	Died
3	44	Died
4	34	Lived
5	44	Died
6	34	Died
7	26	Lived
8	34	Died
9	26	Lived
10	34	Lived
11	44	Died
12	34	Died
13	26	Lived
14	34	Died
15	26	Lived

LC50 calculated according to Dixon and Massey (ref 1, page 437) is 32 mg/m³. However, as explained previously, this is probably erroneously high, since the first MIRAN was calibrated at ambient pressure, while the phosgene level recorded during the experiments were at a pressure of approximately 1.7 PSIG (88 mm HG) due to restrictions in the exposure chamber inlet.

2) Stage II. Examining the need for preventing huddling during exposures.

Phosgene concentrations for stage I are reported as (indicated), uncorrected, inlet MIRAN concentrations. However additional studies showed that the outlet concentrations for the mice exposed *en masse* in groups of 48 were likely approximately 1.2 mg / m³ lower than those exposed divided into groups of 6 to prevent huddling. (See discussion.)

Condition	24-Hour Survival Rate
96 mice exposed *en masse* in groups of 48	69 %
96 mice separated into groups of 6	52 %

(Using Fisher's exact test, two tailed, this difference in survival rates is significant at the p = .027 level of probability)

3) Stage III. Testing for non-uniform exposures in the chamber (96 mice, exposed as groups of 6 in each of the four chamber quadrants - four runs, 24 mice each run)

Reported as survival at 24 hours post exposure to 32 mg/m³ indicated, uncorrected, inlet MIRAN concentration.

Number of survivors at 24 hours post-exposure

		Quadrant: A	B	C	D
Run #:	1	0	1	3	0
	2	5	2	3	1
	3	5	5	5	5
	4	2	5	3	5
Average:		3	3.25	3.5	2.75
Per Cent Survival:		50%	54%	58%	46%

(Chi square analysis indicates no significant difference in survival between quadrants at $p < .05$)

4) Stage IV. Determining the best exposure time for consistent survival rates. (Note - all phosgene concentrations are <u>outlet</u> MIRAN concentrations.)

a) Estimation of the LC50 for 10-minute exposures:

Mouse #	CG concentration mg/m³	Result
1	68	Died
2	52	Lived
3	68	Died
4	52	Died
5	40	Lived
6	52	Died
7	40	Lived
8	52	Lived
9	68	Died

Calculated LC50 is 52 mg/m³

b) Estimation of the LC50 for 30-minute exposures:

Mouse #:	CG concentration: mg/m³	Result:
1	23	Died
2	18	Died
3	14	Lived
4	18	Lived
5	23	Died
6	18	Lived
7	23	Died
8	18	Died
9	14	Died

Calculated LC50 is 16 mg/m³

c) Estimating a best exposure duration time:
(Number surviving each group of 12 mice, compartmented)

Exposure #	10-minute exposures (54 mg/m³)	30-minute exposures (16 mg/m³)
1	7	6
2	9	4
3	11	2
4	9	5
5	10	3
6	5	10
7	4	4
8	10	6
Average survival:	68 %	42 %
Variance:	6.00	6.41

Statistical analysis (F ratio of variances) indicates no significant difference in the variance of the number of survivors between 10 minute and 30 minute exposures at $p < .01$)

5) Other Results:

a) As mentioned previously, the differences in input and output indicated gas concentrations were studied. The following were found (at 32 mg/m³ indicated inlet phosgene concentration).

(1) The apparent drop in phosgene concentration with the chamber empty ranged from 1.5 to 2.3 mg/m³ at 16 to 25 L/min. flow. It was not significantly higher at 25 than at 16 L/min.

(2) The presence of a small amount of water, sodium bicarbonate solution, or alkaline detergent, adsorbed on a paper towel and placed in a beaker in the chamber, produced an additional 2.5 to 3.5 mg/M³ drop (a total of up to 5 mg/m³) inlet to outlet difference).

(3) A single live mouse in the chamber produced a 1 mg/m³ drop in addition to the pressure drop (a total of 1 to 3 mg/m³).

(4) Additional live mice added to the chamber produced total drops of

3 mice -------2.7 mg/m³
6 mice -------2.7 mg/m³
12 mice -------4.2 mg/m³
24 mice -------5.5 mg/m³
48 mice -------7.0 - 10.7 mg/m·

(5) Addition of 12 expired frozen mice to the empty chamber did not increase the apparent drop in concentration over the pressure drop.

(6) The absolute value of the apparent drop in concentration was also proportional to the inlet indicated concentration, as follows (with 24 mice in chamber).

Indicated inlet concentration	Apparent drop in concentration
17 mg/m³	2.5 mg/m³
32 " "	5.5 " "
68 " "	7.7 " "

b) Four mice utilized for rough estimate of 20-minute LC 50, using outlet MIRAN phosgene concentrations

Mouse #	CG concentration mg/m³	Result
1	25	Died
2	21	Died
3	19	Lived
4	21	Lived

(rough estimate of LC 50 is - 21 mg/m³)

c) Average lung weights and lung to body weight ratios of three surviving mice one week post phosgene exposure (note - these mice, as well as their lungs, appeared healthy to inspection.)

Mouse #	Weight (gms.)	Lung Wt. (gms)	Lung to Body Weight Ratio x 100
1	26.5	.2737	1.03
2	29.0	.1974	0.68
3	28.0	.2216	0.79
Average:	27.8	.23	0.83

d) Average lung weights and lung to body weight ratios of four mice that succumbed to phosgene within 24 hours after exposure. (These lungs appeared hemorrhagic and edematous on inspection.)

Mouse #	Weight (gms.)	Lung Wt. (gms)	Lung to Body Weight Ratio x 100
1	24.0	.5098	2.12
2	27.0	.6321	2.34
3	27.0	.4698	1.74
Average:	26.0	.54	2.07

e) Survival times, post exposure to 32 mg/m³ for 20 minutes (indicated inlet concentration).

Total mice observed 7 days	192
Number of mice that survived 5 hours	192
Number of mice that survived 24 hrs	116
Number of mice that survived 7 days	110
Number of mice dying after 24 hrs	6
% of mice dying before 5 hours	0.0 %
% of mice dying between 24 hrs and 7 days	1.5 %

DISCUSSION

Much of inhalation toxicology involves chronic exposures to low concentrations of toxicants. Commercially available chambers are usually designed for subacute and chronic studies. Most are inefficient for acute inhalation exposures.

Dr. A. M. Sciuto developed the whole body exposure module used in these studies. In use, it appears to provide an efficient means of testing treatments for acute toxic lung injury. Before using the chamber for definitive studies, it was necessary to know the characteristics of the exposure chamber when used in the anticipated modes. The results of the studies reported here provide information for use of this exposure chamber, and closely similar chambers, in the future. While some results were essentially as expected, unforeseen results provided additional knowledge for optimum exposure procedures using this and other chambers. The results will be discussed in the order in which they were obtained as the four stages of the protocol were accomplished, followed by a brief discussion of "other results."

Stage I. In determining the LC50 for 20-minute exposures of mice to phosgene we encountered difficulties related to the apparent decrement between the (indicated) inlet and the outlet concentrations of phosgene. We were previously unaware of this decrement, although Silver (ref 6) has previously noted some of these findings. Part of this decrease in apparent phosgene concentration was found to be due to serial pressure drops in the circuit. It is variable in amount, depending on, and proportional to, the flow rate through the circuits. It is due to the use of high flow rates together with (narrow) 1/4 inch tubing and connectors for the outlet of the inlet MIRAN. At the nominal 20 liter per minute flows used for these experiments, pressure in the inlet MIRAN was found to be 1.7 PSIG (88 torr above ambient). The outlet MIRAN has a large diameter (1/2 inch) outlet which causes essentially no pressure buildup at 20 liters per minute flow, and thus avoids the calibration problem entirely. However, an additional, approximately equal, drop in phosgene concentration between the MIRANS was found which appears to be real, possibly due to uptake of phosgene by adsorption or hydrolysis on the chamber walls and pulmonary uptake by the animals themselves This phenomenon in the chamber was capable of producing a drop of 2-3 mg/m³, in the presence of a small amount of liquid in the chamber (and the chamber is almost always wet by animal urine before completion of exposures). An additional drop of up to 5-6 mg/m³ is also found, the magnitude of which is directly but not linearly proportional to the number of animals in the chamber. These findings are similar to those noted by Silver (ref 6) but not further elucidated. They were not

pursued further in our studies because these findings were not anticipated or provided for in formulating the protocol.

Inhalation toxicology study results are usually reported in terms of the gas concentrations to which the test animals are "exposed." The site at which the gas concentrations were measured is rarely specified (e.g., ref 2). This practice is unsatisfactory particularly for whole body exposures, even at the reasonably high flow rates used here. Because of the significant differences between inlet and outlet gas concentrations found, to adequately characterize a gas exposure it is insufficient to specify an inlet concentration of the gas to which the animals are subjected. Some authors advise reporting the gas concentrations obtained by sampling from various sites within the chamber. While at first glance, this may appear a reasonable approach, it is in fact quite unsatisfactory. It leaves undefined just how the exposure concentration is to be denoted in reporting the results for comparison with other results of the original investigator or the results of research by other investigators. If the concentrations obtained vary by location sampled, there is no rationale to support a simple averaging of an arbitrary set of these samples. Likewise, it seems equally arbitrary to report a simple mean of the inlet and the outlet concentrations. A rationale for this would be difficult to establish. The ideal parameter to express would perhaps be the average <u>alveolar</u> gas concentration in the case of a toxic gas known to have its principal effect at the alveolar level. This figure will almost invariably be impractical to determine. In practice, it may be preferable to specify the (time averaged) outlet gas concentration for the exposure, since it reflects the concentration resulting from the changes produced by adsorption, absorption or dissolution of the gas in the chamber.

For studies in which the effect of possible treatments for toxic gas exposures are to be examined, the precise gas concentration obtained is not as critical as is the assurance that treated and control animals receive essentially the same gas concentrations. This can be established by exposing both simultaneously in the same chamber. The use of outlet concentrations to specify the exposure concentrations should be satisfactory in assuring that approximately equal concentrations are established in multiple exposures in spite of varying chamber loads, humidities, and wall conditions.

The (stage I) determination of a 20 minute exposure LC 50 for phosgene was determined using <u>inlet</u> concentration measurements. When the superiority of the <u>outlet</u> concentration measurements became evident, the desirability of repeating the study, using outlet instead of inlet concentrations was considered. However, in considering the large extent to which the variability of the LC50 determinations depend upon specific aspects of each individual study planned, it was felt that the outlet concentration LC50 predicted on the basis of this <u>inlet</u> LC50 is probably sufficiently accurate to serve as the starting point for other projects. It would be difficult to justify additional animals, expense, and time to repeat the studies.

If we speculate that a 5 - 7 mg/m^3 difference in inlet and outlet concentrations obtained (occurred) during the 20 minute up-down LC 50 determinations, we would expect the 32 mg/m^3 inlet LC50 concentration to indicate a 25 to 27 mg/m^3 outlet LC 50 concentration. Two other estimates of the outlet 20-minute LC 50 can be derived from these

studies. They unfortunately vary from the estimate mentioned above. The first is obtained by interpolation from the 10-minute and the 30-minute outlet LC determinations. This interpolation uses Haber's law, which involves interpolation of the inverse functions of the concentration. Using this method, we would predict a 20-minute LC 50 of 24 mg/m^3. The other estimate is based on the brief up-down outlet LC 50 determinations recorded above [page 7, under "5) Other Results (2)"]. There the rough estimate, based on four mice, was 21 mg/m^3. Thus the best estimate we can arrive at is that the 20-minute LC 50 is in the range of these three estimates, namely, 21 to 27 mg/m^3.

Stage II. The study of the effect of exposure in large groups as compared to exposure in small groups is inconclusive, in spite of the significantly greater survival noted in the huddled groups. This is so because of the slightly greater mean phosgene concentration received by the quadrant separated mice. The occurrence of anecdotal reports of suffocation in crowded mice containers suggests that some huddled mice may be hypo-ventilated. This could decrease the pulmonary inhalation of toxic gas and lead to increased survival. This rather than, or in conjunction with, the slightly greater phosgene concentration in the quadrant separated exposures may have provided the greater survival rate in the mice allowed to huddle.

In retrospect, a convincing study of this aspect of whole body exposures would probably require a series of simultaneous exposures of huddled and of separated mice in two individual chambers supplied with phosgene by splitting the incoming gas flow into two streams, one for each chamber. The equality of gas concentrations in the two chambers would then be virtually assured.

Stage III. No evidence for an effect of position in the (four quadrants of the) horizontal plane of the chamber floor was found. An effect of position in the vertical dimension (horizontal stratification) in the chamber is possible. This was not examined here but a previous brief study of rats exposed in this chamber with separation by vertical level found no effect. Currently planned use of the chamber does not require vertical separation of the test mice.

Stage IV. These studies showed equal variances for 10- and 30- minute exposures. Prior to this, it had seemed possible that brief exposures might allow a certain amount of transient breath-holding, in some animals. Dr Bruce Lehnert (ref 3) has recorded minute ventilation of rats during exposures to phosgene, and states that most tend to breath-hold initially. If this is also true of mice, and variable in degree between mice, variable amounts of phosgene might be inhaled during an exposure. This should be reflected in greater variance in survival rates. It was speculated that the effect would be smaller during a 30-minute exposure, in which initial hypo-ventilation would be expected to be balanced to some extent by subsequent compensatory hyperventilation.

The exposure method used in this protocol involved continual on line adjustments of input phosgene concentrations to maintain a steady level of phosgene concentration at the chamber outlet. During short exposures, it is difficult to establish and maintain a steady targeted phosgene level. If the initial target level is overshot or undershot, there is little opportunity for compensatory adjustment of the input concentration before the exposure is completed. Longer exposures allow

a rough averaging of the initial error in reaching the target concentration.

For the above two reasons, it was expected that consistent exposures would be more likely with longer exposures. The results however, show essentially no advantages to longer exposures. This was unexpected, and the explanation is not apparent. In a study of the effect of inhaled formaldehyde vapor on minute ventilation in rats, Chang et al (ref 7) found rats consistently decrease their ventilation as much as 75% for periods as long as 15 minutes. Longer exposures were not examined. If rats are adept at consistently decreasing their ventilation for periods as long as 30 minutes, there may be no advantage in 30-minute as opposed to 10-minute exposure regimens. Considering the practical aspects of exposure routines, our current impression is that a 20-minute exposure provides the optimum balance of speed and convenience with provision for on line adjustment of gas concentrations to average out minor moment to moment variations during the exposures.

Although the LC50 phosgene concentrations initially determined by the up-down method produced a usable approximation to 50 % lethality (58 % lethality for 30 minute exposures, 32 % lethality for 10 minute exposures), in the studies using larger groups of mice the results suggest that a best estimate of the LC 50 for 30 minutes might be a slightly lower concentration of phosgene than that reported here (perhaps 15 mg/m^3) and for the 10-minute LC 50, a slightly higher concentration (perhaps 56 mg/m^3). LC 50 determinations are affected by many variables. While the present determinations offer reasonable starting points for future protocols, they require confirmation and adjustments for specific conditions of each study. Only a rough prediction of exposure concentrations for other lethality ratios, such as LC 75 %, can be made from these results.

Other comments

1) The difference in inlet and outlet indicated concentrations due to pressure buildup in the inlet MIRAN should be avoided by using large diameter (1/2 inch or larger at these flow rates) connections throughout the circuit. The remaining differences, presumably due to absorption or adsorption by the chamber walls and uptake by the animals, probably cannot be avoided without excessively high flow rates in the chamber.

2) The brief 20-minute LC50 determination using four remaining mice was meant to provide a rough check on the two other methods of estimating the outlet phosgene concentration LC50 for 20-minute exposures, as described in the discussion of Stage I results above.

3) The average lung weights and lung to body weight ratios listed here are recorded for reference in future phosgene exposure protocols.

4) The recorded survival times show that almost all deaths from the 20-minute exposures occurred between 5 and 24 hours post exposure. Most of the mice that survived 24 hours appeared quite healthy and active after one week. When the lungs of three of these one-week survivors were examined after euthanasia and necropsy they appeared grossly normal. Observations of the 10-minute and 30-minute exposed mice were not continued past the 24-hour point so that the number of 24-hour survivors that would have died in the following week is not known.

5) The use of a perforated quadrant divider to separate groups of animals during exposure was convenient. A common commercially available white plastic grid material used for recessed lighting louvres provides a surface to which mice find it difficult to cling. Construction of quadrant dividers using this material provides dividers which separate the mice effectively without interfering with gas flows or with the loading and unloading of the chamber with mice. A temporary revolving top incorporating a quadrant shaped door facilitated loading the chamber. It was replaced by the standard chamber top after the chamber was loaded.

CONCLUSIONS

1) The LC 50 for 20-minute exposures of these mice to phosgene, with 24-hour post exposure observation, lies in the range of 21 to 27 mg/m^3 chamber outlet concentration.

2) No conclusion is reached regarding the effect of huddling on gas inhalation toxicity.

3) Location of an animal in any of four (floor) quadrants in this chamber has no significant effect on inhalation exposure survival when compared to any of the other quadrants.

4) Length of exposure, in the range of 10 to 30 minutes, has no significant effect on reproducibility of survival ratios in repeated exposures of different groups of animals to equivalent concentrations of phosgene.

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APPENDIX:

A. A summary of the statistical analyses used in evaluating the results:

Stage I. (and LC 50 determination in Stage IV). No analysis of the accuracy of these LC50 determinations has been made. The accuracy of the up-down procedure is determined by the number of individual up-down runs recorded and by the variance of the number of animals surviving at each concentration under the conditions of the study. The latter variance cannot be estimated accurately from the small number of up-down observations used here.

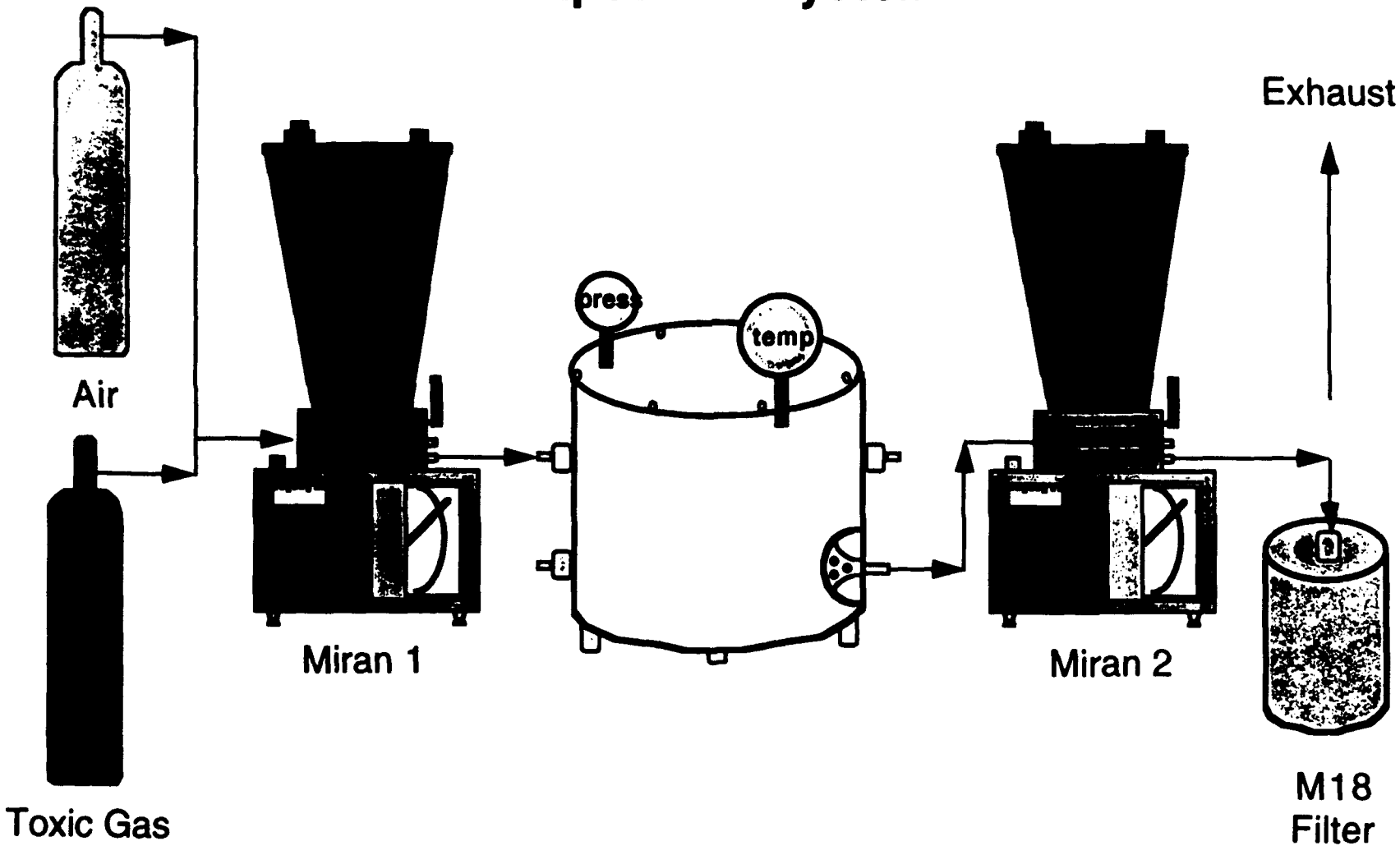
Stage II. Null hypothesis is that there is no difference in the number of mice surviving in the undivided group (66 out of 96 mice) and in the group separated by dividers (50 out of 96). Fishers Exact Test as implemented by the Number Cruncher software program (ref 5) indicates $p = <.0266$. Action, reject hypothesis. However, as pointed out under "Discussion" above, the origin of the difference may have been the slightly higher concentration of phosgene received by the quadrant separated mice.

Stage III. Null hypothesis is that there is no difference between the number of animals surviving in each of the four quadrants. Chi square test for 1 x k (goodness of fit) test was used. Calculated value of Chi Square is 0.4. Critical value of Chi square for $p < 0.05 = 7.82$. Action, accept null hypothesis.

Stage IV. Null hypothesis is that there is no difference in the variances of the number of animals surviving the 10 minute and the 30 minute exposures. The F test for variance ratios (ref 1) was used. F ratio is 1.068. Critical F (double tailed @ $p < .025$) is 0.2. Action, accept null hypothesis.

B. Diagram illustrating the exposure system used in these studies. (diagram appended)

Exposure System



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